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APPLICATION OF PREPARATIVE SCALE SFE/SFC TO FOOD AND NATURAL PRODUCTS

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Summary

The fractionating efficacy of supercritical fluid extraction (SFE) is limited for many applications involving food and natural products. SFE can be combined with preparative supercritical fluid chromatography (SFC) in many cases to enhance the separation between desired solutes and unwanted coextractives for both engineering and analytical applications. Examples will be given on the use of two different modes of SFC: adsorption and size exclusion; to fractionate lipid species, pesticides and environmental contaminants; using such column packings as silica gel, cross-linked divinylbenzene polymers, and n-alkyl silicas. A preparative method for the enrichment and fractionation of tocopherol isomers from seed oil matrices will be presented, which produces results superior to those obtained from SFE alone. Fractionation of target analytes, i.e., pesticides and vitamins from unwanted coextractives, can be achieved using adsorption or size exclusion chromatography with neat SF eluents or conventional liquid mobile phases diluted with SC-CO_2. Finally, an on-line SFE/SFC system will be presented for the extraction and cleanup of foods containing pesticides, prior to their chromatographic analysis.

I. INTRODUCTION

The coupling of supercritical fluid chromatography (SFC) with its extraction analogue (SFE) offers great potential for fractionating complex mixtures which occur in natural materials. Although SFE can accomplish some preliminary fractionation or enrichment of specific components in complex mixtures [1], it is frequently insufficient alone for many separation tasks. The development of tandem SFE/SFC technology is important in order to extend the application of supercritical fluids as benign and environmentally compatible processing agents.

At the USDA's National Center for Agricultural Utilization Research, we have been applying preparative SFC techniques to a broad range of problems in the areas of biomaterial/food processing, as well as analytical chemistry. The definition of the term "preparative" SFC can include a scale embracing both laboratory as well as production scale techniques; however, it can also include the use of the technology for

"preparing" samples for chemical analysis. Actually, both uses of SFC share a common theoretical basis, therefore, results obtained in one area of application can be utilized in the other using similar equipment and column packings.

Previously, we have shown that a low resolution form of SFC can be used to prepare fat-laden food matrices for pesticide residue analysis [2]. The technique which uses silica gel or alumina adsorbents, coupled with supercritical fluid carbon dioxide (SC-CO_2) as an eluent, was successful in fractionating lipid coextractives away from the pesticide fraction. In this presentation, we wish to report on our attempts to extend preparative SFC, coupled with an initial extraction via SFE, to food and natural product mixtures, namely: (a) the fractionation of tocopherol extracts using SFC, (b) the use of SC-CO_2 in place of an organic solvent in size exclusion/adsorption chromatography and (c) the use of microbore SFC as a sample cleanup technique in an on-line SFE/SFC/GC scheme for pesticide residue analysis [3].

II. EXPERIMENTAL

Tocopherols from soybean flakes, rice bran and wheat germ were extracted and chromatographically fractionated using the apparatus previously described by Favati, et al [4]. The selectivity of the SFE step for tocopherols relative to the seed oil was optimized at 25 MPa and 80°C for the soybean flakes. These extraction conditions did not yield the same selectivity for tocopherols when applied to the rice bran and wheat germ matrices. Preparative SFC of the extracts was performed at 25 MPa and 40°C using 16 g of silica gel (60 - 200 mesh, J.T. Baker Chemical Co., Phillipsburg, NJ) contained in a 200 mm X 17 mm (i.d.) column. Fractions of the neat CO_2 eluent were collected every 500L (expanded CO_2 basis) and analyzed for tocopherol and oil content by high performance liquid chromatography (HPLC) and gravimetry, respectively.

The hybrid SFC/HPLC, or enhanced fluidity chromatography [5], experiments were conducted on a Hewlett Packard Model 1082 or 1205 chromatograph (Hewlett-Packard Co., Wilmington, DE). A pressure resistant packing developed for size exclusion chromatography (SEC), Jordi Gel (Jordi Associates, Inc., Bellingham, MA), was used in these modified SFC runs. Jordi Gel consists of a highly cross-linked polymeric resin, 5 u particle size, consisting of 85% divinylbenzene content, which can withstand pressures up to 55 MPa. This packing material can tolerate a variety of organic solvents that were mixed in varying proportions (0 - 100 vol.%) with the SC-CO_2 to form various sub as well as supercritical eluents. Column sizes up to 250 mm X 10 mm (i.d.) were used for generating the experimental data.

The SFE/SFC/GC system was constructed by modifying a Lee Scientific 501 SFC (Dionex Corp., Sunnyvale, CA) and a Milton Roy SPA system (Milton Roy Corp., Riveria Beach, FL). A schematic diagram of the coupled system is shown in Fig. 1. In this case, the recirculation loop for the SF was used to extract the sample from the extractor, resulting in a constant concentration of extracted solute in the circulating SF stream. A six port sampling valve (V2) was then used to divert a portion of the recirculation stream onto a 150 mm X 1 mm (i.d.), 5 μ, octyl Deltabond column (Keystone Scientific, Inc., Bellefonte, PA), functioning as a preparative column for sample cleanup. The sample fractionation is accomplished at 20 MPa and 50°C and

the desired fraction obtained from the SFC eluent by a switching valve, V3. The eluent fraction containing the target analytes is then diverted to a modified injection system on the 501 SFC equipped with a cryotrap. The 501 SFC is then used as an analytical gas chromatograph (GC); the analyte fraction being initially isolated in the cryotrap at 0°C with subsequent desorption at 270°C onto a 30 m X 0.25 mm i.d. fused silica capillary column, having a stationary phase of 95% methyl + 5% phenyl siloxane (J & W Scientific, Folsom, CA). Multiple temperature program runs were optimized for the GC analysis of organochlorine, organophosphorus and carbamate pesticide classes separated on the microbore SFC column [3].

III. RESULTS AND DISCUSSION

a. Preparative SFE/SFC of Tocopherols

Preliminary SFE of tocopherols from seed oil matrices under the above extraction conditions can provide an improved enrichment as shown in Table 1 below. Enrichments of 1.83 to 4.33 over that found in the original seed oil are recorded for the four individual tocopherols after SFE. Transfer of these extracts to the head of the chromatography column and subsequent fractionation provides even further enrichment as noted in Table 1. These enrichments are all expressed in μg-tocopherol/g-oil and are relative to the concentrations of tocopherol found in a petroleum ether-extracted oil sample from the starting flakes. The data show that the delta tocopherol is preferentially enriched with respect to the other isomers.

Mass balance recoveries for two of the tocopherols, gamma and delta, were 62.7 and 60.2%, respectively, from the SFE stage. Recoveries from the SFC step were 75.7 and 87.4% for the gamma and delta isomers, respectively. The inclusion of some unextracted tocopherol residue in the extracted meal and oil provides protection from the deleterious effect of oxidation.

Tocopherol	SFE	SFC
alpha	4.33	12.1
beta	1.83	2.4
gamma	3.94	15.0
delta	3.75	30.8

Table 1: Enrichment of Tocopherols from Soybean Flakes

Analysis of collected fractions taken from the SFC step have indicated that the alpha isomer is isolated in the first fraction, along with a significant quantity of the gamma tocopherol. Fractions 2 and 3 are dominated by the presence of gamma and delta tocopherol, giving way to a preponderance of delta tocopherol in Fractions 4-6. Such results show that tocopherols can be both enriched and selectively fractionated by combining SFE and SFC, although the latter technique is far more effective than SFE alone.

b. Fluid-Liquid Preparative Chromatography

Previously, we have shown that SC-CO_2 can be substituted for methylene chloride using the above size exclusion media for the cleanup of SFE- or solvent-extracted environmental or natural sample matrices [6]. The object of such studies was to minimize the use of methylene chloride, a solvent banned by the Environmental Protection Agency of the USA. Substitution of up to 80% methylene chloride with SC-CO_2 resulted in the retention of the separation pattern of a test mixture consisting of corn oil, a ubiquitous phthalate ester, methoxychlor (a representative pesticide), perylene and sulfur. However, no reduction in the total amount of methylene chloride used was possible due to the excessive long retention times associated with the perylene and sulfur solutes. In order to avoid the use of methylene chloride, we investigated the use of other organic solvents in conjunction with SC-CO_2 as fluid phases for preparative SFC.

As shown in Fig. 2, the dilution of the organic solvent with SC-CO_2 produces significant changes in the resolution and retention of the solutes. For the pure liquid solvent, 100% tetrahydrofuran (THF), the first three peaks are adequately separated; however, the perylene and sulfur are unresolved. However, introduction of 30% of SC-CO_2 into the eluent increases the retention times significantly and enhances the resolution between adjacent peaks, resulting in the resolution of sulfur and perylene. Since the elutropic strength of the solvent is reduced by the introduction of SC-CO_2 at the designated temperature and pressure (13.7 MPa, 40°C), peaks 4 and 5 begin to exhibit some tailing, indicative of adsorption on the hydrophobic resin packing.

Measurements of the void volume, i.e., the exclusion limit and the total permeation limit of the size exclusion column, indicate that there is a 60% loss in pore volume for the 100 A column in going from a 100 to 70% THF eluent. This is due to the introduction of SC-CO_2, which is a poorer swelling solvent than THF. Independent measurements of the swelling of polystyrene/divinylbenzene-based resins [6] indicate that solvents of similar solubility parameter to the styrene or divinylbenzene moieties, i.e., THF, methylene chloride, p-dioxane, and ethyl acetate, increase the pore volume of the resin bead, enhancing it size exclusion capability. For example, peaks 1-3 in Fig. 2 for the 70% THF eluent are well separated; however, with 100% THF in the eluent, the first two peaks are not totally resolved. At leaner THF concentrations, the mechanism of adsorption also begins to predominate as mentioned above. For example, the pesticide methoxychlor (peak 3) is retained by both a size exclusion, as well as adsorption mechanism, since its elution volume exceeds total permeation limit of the SEC column.

The ability to control solute retention and hence resolution by the introduction of a second fluid phase into the liquid eluent offers some unique opportunities for preparative chromatography. Using this principle, the relative contributions of size exclusion and adsorption retention mechanisms can be altered producing higher resolution and separation factors between solute peaks. For example, in Fig. 2, the separation factor, alpha, between peaks 3 & 4 is increased from 1.48 at 100 % THF to 2.07 for 70% THF in the eluent. This creates quite a large retention interval for separating different generic classes of solutes, i.e., pesticides, vitamins, etc. from other

lipid moieties. We have currently been exploiting this retention phenomena in the application of fluid-liquid chromatography for sample preparation for pesticide residue and nutrient analysis.

c. Microbore SFC for Sample Cleanup

Using the above described SFE/SFC/GC system, we have successfully analyzed several classes of pesticides, both incurred as well as spiked, in meat samples. The key to the efficacy of this on-line analysis method is the use of a microbore SFC column to "prepare" the sample for subsequent GC analysis. Failure to retain or fractionate unwanted fat in the SFC step would be extremely deleterious to the GC column.

To illustrate the efficacy of the above system, 12 organochlorine pesticides were spiked in poultry fat, extracted by SFE under the same conditions as used for the SFC step. Recoveries ranged from 84-125% for analyte concentrations of 4-68 ppb in the fat matrix. These concentrations are all under the minimum detection limits required by FSIS, the major regulatory agency in the USA, charged with the inspection of meat products [7]. A similar analysis of actual incurred residues in chicken adipose tissue gave excellent agreement with results obtained from off-line analysis of these identical samples, as shown in Table 2. These results are even more remarkable when one considers that over a year had elapsed between the on-line and off-line analysis.

Pesticide	On-Line SFE/SFC/GC	Off-Line
Heptachlor Epoxide	0.49 ppm	0.56 ppm
Dieldrin	1.75 ppm	2.33 ppm
Endrin	2.03 ppm	2.00 ppm

Table 2: Comparison of On- and Off-Line SFE Analysis for Incurred Pesticide Residues in Poultry Tissue.

Additional experiments on poultry fat spiked with organophosphorus pesticides were also successful using the microbore SFC column as a preliminary cleanup step. Detection limits between 6-22 ppb were recorded for six different P- containing pesticides via nitrogen/phosphorus detection. For poultry fat, analyte recoveries were between 78-104%; for ground beef, 79-94%; and for spiked lard, 82-103%. The tabulated recoveries are certainly acceptable considering the ppb concentrations of the organophosphorus in the fatty meat matrices. The described on-line method saves time and the use of organic solvent, and can be readily automated.

IV. CONCLUSIONS

The above examples clearly illustrate the advantages of using preparative SFC in process as well as analytical applications. It should be appreciated that a synergism exists between sample preparation methods using SFC and the utilization of these same

concepts in preparative or production chromatography. For example, the use of highly cross-linked pressure resistant polymeric packings that are compatible with supercritical fluid media opens up a number of process applications as well as the analytical application noted in this study.

ACKNOWLEDGEMENT

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DISCLAIMER

The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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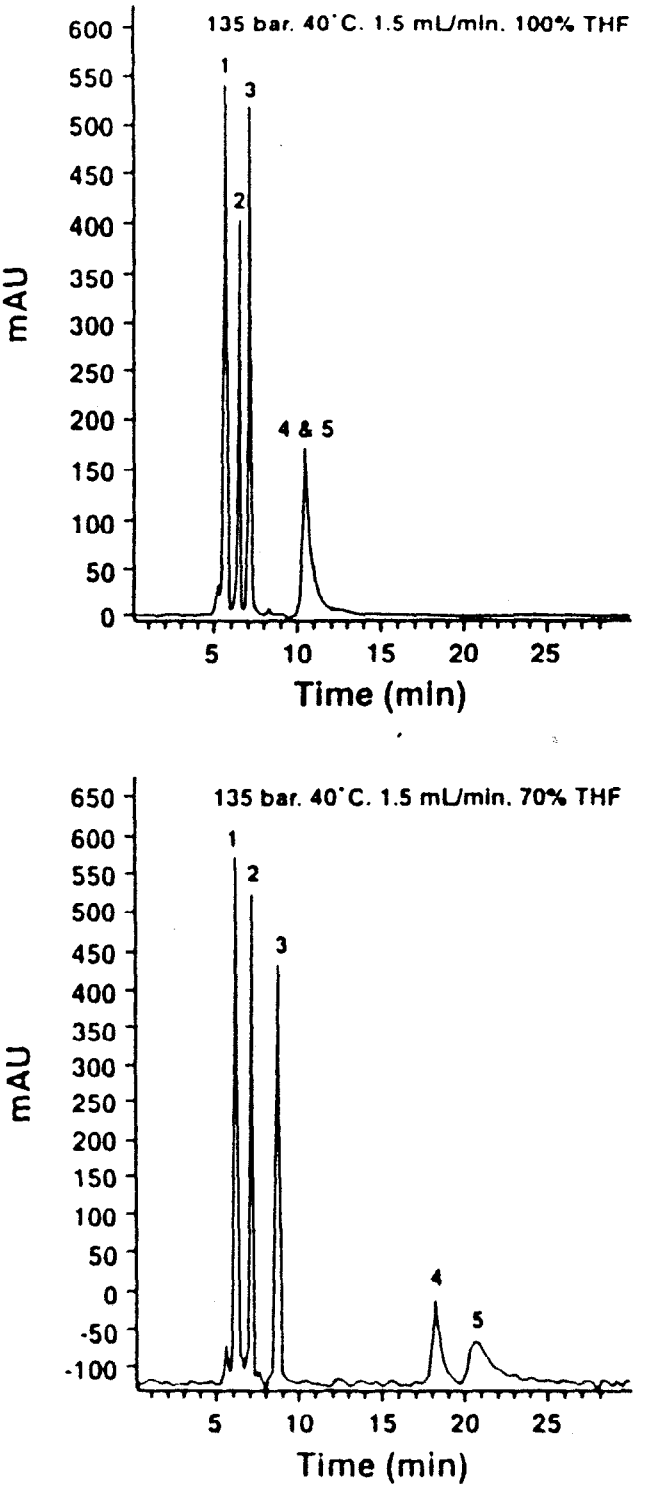

Figure 2: Effect of Dilating the Liquid Mobile Phase (THF) with SC-CO_2 on the Resultant Separation of Corn Oil (1), bis(2-Ethylhexyl) Phthalate (2), Methoxychlor (3), Perylene (4) and Sulfur (5).